WHAT IS CLAIMED:

1. A method for preparing adenovirus particles from an adenovirus preparation comprising the steps of:

(a) subjecting said adenovirus preparation to chromatography on a first chromatographic medium, whereby adenovirus particles from said adenovirus preparation are retained on said first chromatographic medium;

(b) eluting adenovirus particles from said first chromatographic medium to produce an eluate of adenovirus particles;

(c) subjecting adenovirus particles from said eluate to chromatography on a second chromatographic medium, wherein said second chromatographic medium retains one or more contaminants from said eluate and wherein said second chromatographic medium is not solely a size exclusion medium; and

(d) collecting adenovirus particles from said eluate.

2. The method of claim 1, wherein said first chromatographic medium is selected from the group consisting of an anion exchange medium, cation exchange medium, immobilized metal affinity medium, sulfated affinity media, immunoaffinity medium, heparin affinity medium, hydroxyapetite medium and hydrophobic interaction medium.

3. The method of claim 2, wherein said first chromatographic medium is an anion exchange medium.

4. The method of claim 3, where the anion exchange medium is Amersham Biosciences Source 15Q.

5. The method of claim 1, wherein said second chromatographic medium is selected from the group consisting of cation exchange media, anion exchange media, immobilized metal affinity media, sulfated affinity media, dye affinity media, hydroxyapetite media, immunoaffinity media, heparin affinity media and hydrophobic interaction media.

6. The method of claim 5, wherein said second chromatographic medium is dye affinity media.

7. The method of claim 6, wherein said dye affinity media is BioSepra Blue Trisacryl.

8. The method of claim 6, wherein said dye affinity media comprises an agarose-based support matrix that is crosslinked to about 6%.

9. The method of claim 1, wherein the adenovirus preparation is prepared from host cells.

10. The method of claim 9, wherein the host cells are capable of complementing replication.

11. The method of claim 1, wherein said adenovirus preparation comprises an adenoviral vector encoding an exogenous gene construct.

12. The method of claim 11, wherein said exogenous gene construct encodes a therapeutic gene.

13. The method of claim 12, wherein said therapeutic gene encodes antisense ras, antisense myc, antisense raf, antisense erb, antisense src, antisense fms, antisense jun, antisense trk, antisense ret, antisense gsp, antisense hst, antisense bcl antisense abl, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV ras, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11 IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24; IL-25, IL-26, MDA-7, PTEN, Interferon-α, Interferon-β, Interferon-γ, α-fetoprotein, GM-CSF, G-CSF, thymidine kinase, p53 or other gene selected from the group consisting of the genes listed in Table A.

14. The method of claim 13, wherein said therapeutic gene encodes p53.

15. The method of claim 11, wherein said gene construct is operatively linked to a promoter.

16. The method of claim 15, wherein said promoter is SV40 IE, RSV LTR, β-actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

17. The method of claim 9, wherein the host cells are 293 cells.

18. A method for preparing adenovirus particles from an adenovirus preparation comprising the steps of:

(a) subjecting said adenovirus preparation to chromatography on a first chromatographic medium, whereby contaminants from said adenovirus preparation are retained on said first chromatographic medium;

(b) subjecting adenovirus particles remaining in the eluant to chromatography on a second chromatographic medium whereby adenovirus particles from said eluant are retained on said second chromatographic medium, wherein when said second chromatographic medium is an anion exchange medium, then said first chromatographic medium is a medium other than a sulfonated polysaccharide affinity medium, and

(c) eluting adenovirus particles from said second chromatographic medium.

19. The method of claim 18, wherein said first chromatographic medium is selected from the group consisting of cation exchange media, anion exchange media, immobilized metal affinity media, sulfated affinity media, dye affinity media, hydroxyapetite media, immunoaffinity media, heparin affinity media and hydrophobic interaction media.

20. The method of claim 19, wherein said first chromatographic medium is a dye affinity media.

21. The method of claim 19, wherein said dye affinity media is BioSepra Blue Trisacryl.

22. The method of claim 20, wherein said dye affinity media comprises an agarose-based support matrix that is crosslinked to about 6%.

23. The method of claim 18, wherein said second chromatographic medium is selected from the group consisting of anion exchange media, cation exchange media, immobilized metal affinity media, sulfated affinity media, immunoaffinity media, heparin affinity media, hydroxyapetite media and hydrophobic interaction media.

24. The method of claim 23, wherein said second chromatographic medium is an anion exchange medium.

25. The method of claim 24, wherein said anion exchange medium is Amersham Biosciences Source 15Q.

26. A method for preparing adenovirus particles from an adenovirus preparation comprising the steps of:

(a) subjecting said adenovirus preparation to chromatography on a first chromatographic medium, whereby contaminants from said adenovirus preparation are retained on said first chromatographic medium;

(b) subjecting adenovirus particles remaining in the eluant to chromatography on a second chromatographic medium whereby further contaminants are retained on said second chromatographic medium; and

(c) collecting the adenovirus particles remaining in the eluant after step (b).

27. The method of claim 26, wherein said first and second chromatographic medium are different.

28. The method of claim 26, wherein said first chromatography medium is not a sulfonated polysaccharide affinity medium and said second chromatographic medium is not an anion exchange medium.

29. The method of claim 27, wherein said first chromatographic medium is selected from the group consisting of cation exchange media, anion exchange media, immobilized metal affinity media, sulfated affinity media, dye affinity media, hydroxyapetite media, immunoaffinity media, heparin affinity media and hydrophobic interaction media.

30. The method of claim 29, wherein said first chromatographic medium is a dye affinity media.

31. The method of claim 30, wherein said dye affinity media is BioSepra Blue Trisacryl.

32. The method of claim 30, wherein the dye affinity media comprises an agarose-based support matrix that is crosslinked to about 6%.

33. The method of claim 27, wherein said second chromatographic medium is selected from the group consisting of cation exchange media, anion exchange media, immobilized metal affinity media, sulfated affinity media, dye affinity media, hydroxyapetite media, immunoaffinity media, heparin affinity media and hydrophobic interaction media.

34. The method of claim 33, wherein said second chromatographic medium is a heparin affinity media.

35. The method of claim 33, wherein said heparin affinity media comprises an agarose-based support matrix that is crosslinked to about 6%.

36. A method for preparing adenovirus particles from an adenovirus preparation comprising the steps of:

(a) subjecting said adenovirus preparation to chromatography on a first chromatographic medium, whereby adenovirus particles from said adenovirus preparation are retained on said first chromatographic medium;

(b) eluting adenovirus particles from said first chromatographic medium to produce a first eluate of adenovirus particles;

(c) subjecting said first eluate of adenovirus particles to chromatography on a second chromatographic medium, whereby adenovirus particles from said first eluate are retained on said second chromatographic medium, wherein

when said first chromatographic medium is an anion exchange medium, said second chromatographic medium is a medium other than immobilized metal affinity medium, a size exclusion medium, anion exchange medium, cation exchange medium or hydrophobic interaction medium;

(d) eluting adenovirus particles from said second chromatographic medium to produce a second eluate of adenovirus particles; and

(e) collecting adenovirus particle from said second eluate.

37. The method of claim 36, wherein said first chromatographic medium is selected from the group consisting of anion exchange medium, cation exchange medium, immobilized metal affinity medium, sulfated affinity medium, immunoaffinity medium, heparin affinity medium, hydroxyapetite medium and hydrophobic interaction medium.

38. The method of claim 37, wherein said first chromatographic medium is an anion exchange medium.

39. The method of claim 38, wherein said anion exchange medium is Amersham Biosciences Source 15Q.

40. The method of claim 36, wherein said second chromatographic medium is selected from the group consisting of anion exchange medium, cation exchange medium, immobilized metal affinity medium, sulfated affinity medium, immunoaffinity medium, heparin affinity medium, hydroxyapetite medium and hydrophobic interaction medium.

41. The method of claim 40, wherein said second chromatographic medium is an immunoaffinity medium.

42. The method of any one of claims 1, 18, 26 or 36, wherein said adenovirus preparation is prepared according to a method comprising the steps of:

a) growing host cells in cell culture media;

b) providing nutrients to said host cells by perfusion, fed-batch, bioreactor, or automated roller bottles;

c) infecting said cells with an adenovirus; and

d) lysing said host cells to provide a cell lysate comprising said adenovirus preparation.

43. The method of claim 42, wherein said cell culture media is serum free media.

44. The method of claim 42, wherein said host cells are grown in a bioreactor.

45. The method of claim 42, wherein said host cells are grown on microcarriers.

46. The method of claim 42, wherein said cell culture media comprises glucose.

47. The method of claim 46, wherein the cells are perfused in said media at a rate to provide a glucose concentration of between about 0.7 and 1.7 g/L.

48. The method of claim 42, wherein the lysis method is a method selected from the group consisting of hypotonic solution, hypertonic solution, impinging jet, microfluidization, solid shear, detergent, liquid shear, high pressure extrusion, autolysis and sonication.

49. The method of claim 48, wherein the cells are lysed by detergent lysis.

50. The method of claim 49, wherein the cells are lysed by detergent Thesit®, NP-40®, Tween-20®, Brij-58®, Triton X-100® or octyl glucoside.

51. The method of claim 50, wherein said detergent is present in the lysis solution at a concentration of about 1% (w/v).

52. The method of claim 42, wherein said lysate is subjected to a diafiltration step.

53. The method of claim 42, further comprising the step of treating the lysate with a nuclease to reduce the concentration of contaminating nucleic acid.

54. The method of claim 42, further comprising the steps of concentrating said cell lysate, exchanging buffer of said cell lysate, and reducing the concentration of contaminating nucleic acids in said cell lysate.

55. The method of any one of claims 1, 18, 26 or 36 wherein the chromatography steps are carried out at a pH range of between about 7.0 and about 10.0.

56. The method of any one of claims 1, 18, 26 or 36 wherein the recovery of purified adenovirus after the second chromatography step is 70% ±10% of the starting PFU.

57. The method of claim 42, further comprising a concentration step employing membrane filtration.

58. The method of claim 57, wherein said filtration is tangential flow filtration.

59. The method of claim 57, wherein said filtration utilizes a 100 to 300K NMWC, regenerated cellulose, or polyether sulfone membrane.

60. The method of claim 42, wherein said media is a serum-free media and said host cells are capable of growing in serum-free media.

61. The method of claim 60, wherein said host cells have been adapted for growth in serum-free media by a sequential decrease in the fetal bovine serum content of the growth media.

62. The method of claim 42, wherein said cells are grown as a cell suspension culture.

63. The method of claim 42, wherein said cells are grown as an anchorage-dependent culture.

64. The method of claim 42, wherein the nutrients are provided by a fed-batch process.

65. The method of claim 42, wherein the nutrients are provided by perfusion.

66. A method for preparing adenovirus particles from an adenovirus preparation comprising the steps of:

(a) preparing said adenoviral preparation according to a method comprising the steps of:

i) growing host cells in cell culture media

ii) providing nutrients to said host cells by perfusion, fed-batch, bioreactor, or automated roller bottles;

iii) infecting said cells with an adenovirus; and

iv) lysing said host cells to provide a cell lysate comprising said adenovirus preparation;

(b) subjecting said adenovirus preparation to chromatography on a first chromatographic medium, whereby adenovirus particles from said adenovirus preparation are retained on said first chromatographic medium;

(c) eluting adenovirus particles from said first chromatographic medium to produce an eluate of adenovirus particles;

(d) subjecting adenovirus particles from said eluate to chromatography on a second chromatographic medium, wherein said second chromatographic medium retains one or more contaminants from said eluate and wherein said second chromatographic medium is not solely a size exclusion medium; and

(e) collecting adenovirus particles from said eluate.

67. A method for preparing adenovirus particles from an adenovirus preparation comprising the steps of:

(a) preparing said adenoviral preparation according to a method comprising the steps of:

i) growing host cells in cell culture media

ii) providing nutrients to said host cells by perfusion, fed-batch, bioreactor, or automated roller bottles;

iii) infecting said cells with an adenovirus; and

iv) lysing said host cells to provide a cell lysate comprising said adenovirus preparation;

(b) subjecting said adenovirus preparation to chromatography on a first chromatographic medium, whereby contaminants from said adenovirus preparation are retained on said first chromatographic medium;

(c) subjecting adenovirus particles remaining in the eluant to chromatography on a second chromatographic medium, whereby adenovirus particles from said eluant are retained on said second chromatographic medium, wherein said first chromatographic medium is a medium other than a sulfonated polysaccharide affinity medium when said second chromatographic medium is an anion exchange medium; and

(d) eluting said adenovirus particles from said second chromatographic medium.

68. A method for preparing adenovirus particles from an adenovirus preparation comprising the steps of:

(a) preparing said adenoviral preparation according to a method comprising the steps of:

i) growing host cells in cell culture media

ii) providing nutrients to said host cells by perfusion, fed-batch, bioreactor, or automated roller bottles;

iii) infecting said cells with an adenovirus; and

iv) lysing said host cells to provide a cell lysate comprising said adenovirus preparation;

(b) subjecting said adenovirus preparation to chromatography on a first chromatographic medium, whereby contaminants from said adenovirus preparation are retained on said first chromatographic medium;

(c) subjecting adenovirus particles remaining in the eluant to chromatography on a second chromatographic medium whereby further contaminants are retained on said second chromatographic medium; and

(d) collecting the adenovirus particles remaining in the eluant after step (c).

69. A method for preparing adenovirus particles from an adenovirus preparation comprising the steps of:

(a) preparing said adenoviral preparation according to a method comprising the steps of:

i) growing host cells in cell culture media

ii) providing nutrients to said host cells by perfusion, fed-batch, bioreactor, or automated roller bottles;

iii) infecting said cells with an adenovirus; and

iv) lysing said host cells to provide a cell lysate comprising said adenovirus preparation;

(b) subjecting said adenovirus preparation to chromatography on a first chromatographic medium, whereby adenovirus particles from said adenovirus preparation are retained on said first chromatographic medium;

(c) eluting adenovirus particles from said first chromatographic medium to produce a first eluate of adenovirus particles;

(d) subjecting said first eluate of adenovirus particles to chromatography on a second chromatographic medium, whereby adenovirus particles from said first eluate are retained on said second chromatographic medium, wherein when said first chromatographic medium is an anion exchange medium, then said

second chromatographic medium is a medium other than an immobilized metal affinity medium, a size exclusion medium, an anion exchange medium, a cation exchange medium or a hydrophobic interaction medium;

(e) eluting adenovirus particles from said second chromatographic medium to produce a second eluate of adenovirus particles; and

(f) collecting adenovirus particle from said second eluate.

70. An adenovirus preparation produced by a method according to any of claims 1, 18, 26, 36, 66, 67, 68, or 69.

71. An adenovirus preparation produced by a method according to any of claims 1, 18, 26, 36, 66, 67, 68, or 69, wherein the preparation is substantially pure.

72. The adenovirus preparation of claim 71, wherein said adenovirus preparation is about 98% pure.

73. The adenovirus preparation of claim 71, wherein bovine serum albumin is present from about 0.1% or less by weight based on the total weight of the composition.